Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus


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The susceptibility of monocyte-derived immature dendritic cells (DC) to infection by various strains of human cytomegalovirus (HCMV) was analysed. Immature DC were generated by incubation of peripheral blood monocytes with interleukin-4 and granulocyte-macrophage colony-stimulating factor for 7 days and were characterized by a CD1a+/CD40+/CD80+/HLA-DR+/CD14− phenotype. Viral antigen expression and production of infectious progeny virus were analysed in infected immature DC cultures. Immature DC were 80–90 % susceptible to HCMV strains that had been propagated in endothelial cell culture, whereas the infection rate was negligible with fibroblast-adapted HCMV strains. Immature DC infection resulted in expression of viral immediate early, early and late genes. Productive infection was proven by the detection of infectious virus in single-step growth curves and in infectious centre assays. It is concluded that HCMV might interfere with the host immune reaction by permissive, lytic infection of immature DC.

Dendritic cells (DC) are antigen-presenting cells that are ubiquitously distributed in virtually all organ systems in the human body (Hart, 1997). DC are assumed to pick up antigens in the periphery and carry them to lymphatic tissues via the afferent lymph vessels (Cella et al., 1997; Hart, 1997). After differentiation into mature DC, they are most efficient in presenting antigens to lymphocytes through the MHC I and MHC II pathway (Cella et al., 1997; Hart, 1997). DC play a central role during virus infections. They may distribute virus from peripheral entry sites towards lymphatic tissues; and infection of DC may pivotally result either in immunosuppression or in immune activation, depending on the cytopathic effect (CPE) of the virus (Bender et al., 1998; Fugier Vivier et al., 1997; Grosjean et al., 1997; Kaiserlian et al., 1997; Klagge & Schneider-Schaulies, 1999). Human cytomegalovirus (HCMV) is a β-herpesvirus with a broad target cell range that causes disseminated infection with replication in numerous organs (Sinzger et al., 1995). In the immunocompromised host, clinical manifestations like interstitial pneumonitis, retinitis, hepatitis and gastrointestinal disease, with significant morbidity and mortality, can occur. In allogeneic bone marrow transplant recipients, delayed reconstruction of the cellular immunity was found to be associated with HCMV infection (Einsele et al., 1993), indicating a possible interaction of HCMV with components of the immune system. In cell culture systems, susceptibility of bone marrow stromal cells and hematopoietic cells to HCMV infection has been reported (Torok Storb et al., 1993). Macrophages were shown to be permissively infected in vitro and in vivo (Ibanez et al., 1991; Lathey & Spector, 1991; Minton et al., 1994; Sinzger et al., 1996). In contrast, little is known about the susceptibility of DC to HCMV infection. Though HCMV seems to be latent in precursors of DC (Hahn et al., 1998), monocyte-derived immature DC have been reported to be non-permissive for HCMV infection (Soderberg-Naucler et al., 1998). However, as has been shown for endothelial cells (EC) and macrophages (Minton et al., 1994; Sinzger et al., 1997; Waldman et al., 1991), strong interstrain differences may also exist regarding the efficiency of infection of DC. We have investigated the hypothesis that certain HCMV variants may cause efficient infection of cultured DC.

Immature DC were generated from adherent peripheral blood mononuclear cells (PBMC) as recently described (Brossart & Bevan, 1997; Sallusto & Lanzavecchia, 1994). Briefly, peripheral blood was obtained from HCMV-seropositive donors. PBMC were prepared by centrifugation on a Ficoll–Hypaque (Lymphoprep; Nycomed) density gradient, resuspended in RPMI containing 10% FCS, 2.4 mmol/l glutamine and 100 µg/ml gentamicin, and allowed to adhere to 6-well tissue culture dishes. After 2 h at 37 °C in 5% CO₂ atmosphere, the non-adherent cells were removed. The adherent fractions were cultured in RPMI–10% FCS (Gibco), supplemented with 1000 U/ml interleukin-4 (IL-4; Genzyme) and 100 ng/ml granulocyte–macrophage colony-stimulating...
factor (GM-CSF; Leukomax, Sandoz) on the initial day of culture. Cytokines were replenished on days 2, 4 and 6. During this incubation period a homogeneous non-adherent cell population with typical immature DC morphology developed (Fig. 1b). On day 7, non-adherent cells were collected by moderate aspiration and transferred to fresh 6-well plates. These cells consisted of 90% immature DC as determined by the CD1a+ /CD40+ /CD80+ /CD86+ /HLA-DR+ /CD14− phenotype (Fig. 1a) and were used for all experiments described. The phenotype of these immature DC was further
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Fig. 2. Kinetics of HCMV antigen expression in infected DC. Detection of (a) IE antigen (MAb E13, UL122/123) in DC 1 day after infection, (b) early antigen p52 (MAb BS510, UL44), (c) late antigen MCP (MAb 28-4, UL86), (d) late antigen pp150 (MAb XP1, UL32) in DC 5 days after infection, and (e) late antigen pp150 in mock-infected cells 5 days after mock-infection by indirect immunoperoxidase technique; bar, 50 µm. (f) Time-course of appearance of viral antigens in infected DC.

confirmed by their ability to differentiate into mature DC when TNFα was added after 7 days incubation with IL4 + GM-CSF (Brossart et al., 1998).

To analyse the susceptibility of immature DC for HCMV, cells were infected with various HCMV strains. Fibroblast-adapted strains AD169 and VHL/F as well as EC-adapted strains TB40/E and VHL/E were included. Strains VHL/F and VHL/E were generated by 20 passages of a recent isolate in fibroblasts and EC, respectively (Waldman et al., 1991). TB40/E was propagated in our group by passaging of a recent clinical isolate on EC. Cells were infected by overnight incubation with cell-free virus preparations at 37 °C at an m.o.i. of 1. For high titre virus preparations (m.o.i. fl50), 100 ml cell-free supernatant of infected HFF cultures was concentrated by ultracentrifugation at 80,000 g for 70 min and pelleted virus particles were resuspended in 1 ml of the respective media. After 24 h infection, cells were fixed in methanol/acetone (1:1) and HCMV immediate-early (IE) antigen was detected on cytospin preparations in the nuclei of infected immature DC by indirect immunoperoxidase staining using MAb E13 (pUL122/123; Biosoft), peroxidase-conjugated goat antimouse IgG antiserum and diaminobenzidine as chromogen. Infected cells were identified as DC by simultaneous detection of DC marker CD1a (MAb anti-CD1a; DAKO) using indirect immunoalkaline phosphatase staining with Fast Red as chromogen. EC-adapted HCMV variants TB40/E and VHL/E infected immature DC more efficiently than the fibroblast-adapted strains AD169 and VHL/F. In low-dose infection experiments (m.o.i. = 1) with VHL/F, < 1% IE antigen expression could be detected in immature DC. In contrast, HCMV strains TB40/E and VHL/E infected 10 and 20% of immature DC, respectively. The efficiency of infection could be further enhanced by using higher infectious titres. At an m.o.i. of 50, 80 and 90% of immature DC were infected with EC-adapted HCMV strains TB40/E (Fig. 1f) and VHL/E, respectively. In contrast, fibroblast-adapted strains AD169 (Fig. 1e) and VHL/F infected immature DC with dramatically decreased efficiency (< 1%), even at an m.o.i. of 50. These differences were not reflected when pp65 (UL83, MAb 28-77; kindly provided by W. Britt, Department of Pediatrics, University of Alabama, Birmingham, AL, USA) was detected by an immunoperoxidase technique in infected cells early after infection as a marker of virus entry (data not shown), indicating that interstrain differences occurred subsequently to penetration of virus particles. Though the exact mechanism underlying these interstrain differences still has to be determined, our results indicated that immature DC are readily susceptible to HCMV infection only with EC-adapted HCMV strains. Following infection with these virus strains, immature DC became enlarged and displayed nuclear inclusions representing late stage CPE (Fig. 1c, d). In addition, infected cells loosely re-adhered and formed syncytia.

We next examined whether immature DC infected by HCMV strains TB40/E and VHL/E were permissive for the
complete virus replication cycle. We analysed IE, early and late antigen expression in immature DC at various intervals after infection. In particular, we detected the IE1 and IE2 antigens (UL122/123; Biosoft), the early antigen p52 (UL44, MAb BS510; Biotest), and the late viral proteins pp150 (UL32, MAb XP1; Behringwerke) and major capsid antigen (MCP, UL86, MAb 28-4; kindly provided by W. Britt) in cytospin preparations by an indirect immunoperoxidase technique (Fig. 2). Antigens of all phases of HCMV replication were detectable in up to 93% of cells. All antigens occurred with typical localization but with slightly delayed kinetics as compared to standard fibroblast cultures. IE antigens were detectable from day 1 after infection, early antigen was detectable from day 2 after infection, and late antigen was detectable from day 3 after infection. At that time-point, CPE occurred in infected cultures, namely re-adherence of the cells, formation of syncytia, and formation of nuclear inclusions. Starting on day 0–8 after infection, the number of viable cells decreased significantly and cell detritus appeared. Lysis of the infected cultures was complete on day 12 after infection. Cell lysis appeared to be caused by HCMV infection, since lysis of DC cultures was not detected during 12 days after mock-infection. In summary, these experiments demonstrated that immature DC were permissive to the complete virus replication cycle and that infection was cytopathic and lytic.

Finally, we sought to determine whether late-stage-infected immature DC produced infectious particles. We performed single-step growth curves to analyse whether infectious virions were produced in and released from infected cells. Immature DC cultures were infected with HCMV strains TB40/E and VHL/E at an m.o.i. of 1. After 24 h incubation, cultures were washed and treated with trypsin to remove residual input infectivity. Supernatants of infected cultures as well as ultrasonic cell lysates were collected daily for determination of the titre of infectious HCMV by limiting dilution analysis. Briefly, the infectious titre was determined by detection of IE antigens after 24 h infection with serial dilutions in fibroblasts. Production of cell-associated virus was first detected on day 4 after infection (Fig. 3 a). The release of infectious HCMV started on day 5 (TB40/E) or day 6 (VHL/E, Fig. 3 a) after infection and reached a maximum level on day 6 (TB40/E) or day 8 (VHL/E, Fig. 3 a). A significant 4-3 log step rise of infectivity was measured in the supernatant of cultures infected with TB40/E and VHL/E. Although these growth curves already indicated productive infection of immature DC, we sought to formally exclude the theoretical possibility that infectivity may result from contaminating cells rather than from infected immature DC. To formally prove on a single cell level that infectious virus is actually released by infected immature DC, we performed infectious centre assays. This assay detected whether late-stage-infected immature DC were capable of transmitting HCMV to co-cultured indicator cells. Six days after infection of immature DC with TB40/E and VHL/E at an m.o.i. of 1, infected cells were sparsely seeded in co-culture with abundant non-infected fibroblasts. After an additional day in co-culture, cells were fixed with 80% acetone and viral antigens were detected by indirect immunoperoxidase staining. Late viral antigen was detected only in the centre of infectious foci (Fig. 3 d). Late-stage-infected cells were identified as DC by immunostaining with an HLA class II antibody (anti HLA-DR; DAKO) (Fig. 3 d) and CD 1a (data not shown). IE viral antigen was also expressed by the surrounding fibroblasts (Fig. 3 b, c). Obviously, late-stage-infected DC transmitted infectious virus to adjacent fibroblasts resulting in foci of infected cells. To formally exclude the possibility that this might be caused by residual input virus, we did control experiments with 24 h-infected immature DC instead of late-stage-infected immature DC. Only late-stage-infected immature DC [6 days post-infection (p.i.)] formed infectious foci in fibroblast cultures, while immature DC at 24 h p.i. did not (Fig. 3 e). This proved that infectious foci were not caused by residual input virus but by de novo synthesized virus progeny.

Taken together, these results demonstrate that EC-adapted HCMV strains are highly efficient in infecting immature DC and that the course of infection appears to be productive and lytic. In contrast, fibroblast-adapted HCMV strains failed to cause significant cytopathogenicity in immature DC. Recently, immature DC generated by the same protocol were reported to be non-permissive for HCMV replication (Soderberg-Naucler et al., 1998). Despite a possible contribution of minor differences in cell culture conditions, the most probable explanation of these differing findings is the use of different virus strains. When cells were infected with fibroblast-adapted strains, our findings were concordant with the data reported by Soderberg-Naucler et al. (1998). Only when immature DC were infected with EC-propagated strains did efficient productive infection occur. In addition, it would be interesting to evaluate the potential of recent clinical isolates to infect immature DC. However, these isolates did not produce significant titres of infectious virus prior to passage 10–20 and could therefore not be included in the study. Still, our finding of dramatic interstrain differences in the infection efficiency of immature DC underscores the importance of strain differences in the understanding of HCMV biology. Recently, a 13 kb deletion of HCMV strain AD169 as compared to clinical HCMV isolates has been reported and subsequently has been suggested as an explanation for interstrain differences in the biology of HCMV (Cha et al., 1996; Kemble et al., 1996). In this study, the presence of the 13 kb gene region did not correlate with interstrain differences in the infection of immature DC as both the non-DC-tropic strain VHL/F and the DC-tropic strains VHL/E and TB40/E contain this gene region (data not shown).

If interstrain differences in the infection of DC also applied to the in vivo situation, this would provide one explanation for the highly variable course of HCMV infections in the host. In terms of virus pathogenesis, this may include a variable degree
of primary replication in DC at the sites of virus entry to mucosal tissues, a variable degree of virus dissemination by infected DC via the lymph, and a variable degree of interaction with immune functions of DC. HCMV infection of DC could have different effects on the immune function of DC. Whereas efficient presentation of viral antigens has been reported for DC infected by influenza virus (Bender et al., 1998; Bhardwaj et al., 1994) or replication-deficient adenovirus (Brossart et al., 1997), measles virus caused suppression of immune functions in infected DC (Fugier Vivier et al., 1997; Grosjean et al., 1997; Kaiserlian et al., 1997; Schnorr et al., 1997). As down-regulation of the MHC I pathway has been demonstrated in HCMV-infected fibroblasts (Hengel et al., 1995; Steinmassl & Hamprecht, 1994), it will be interesting to analyse whether the same effect occurs in infected DC. In this context, the dramatic interstrain differences that we found regarding efficiency of immature DC infection may be relevant. While the expression of gene products down-regulating MHC I or II (Ahn et al., 1996; Fruh et al., 1997; Hengel et al., 1996; Jones et al., 1996; Lehner et al., 1997; Machold et al., 1997; Miller et al., 1998; Wiertz et al., 1996) is likely to occur during lytic infection of DC, such HCMV variants that cannot initiate viral gene expression in DC will most likely not interfere with the immune functions of these cells. These considerations are hypothetical, but the cell culture model presented here will enable future analyses of the functional effects of HCMV variants on infected immature DC.

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References


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